# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)



| Applicant's or agent's file reference P103734WO | **FOR FURTHER ACTION** | See Form PCT/IPEA/416 |
|---|---|---|
| International application No. PCT/GB2004/003492 | International filing date *(day/month/year)* 13.08.2004 | Priority date *(day/month/year)* 10.12.2003 |
| International Patent Classification (IPC) or national classification and IPC C07K14/47 | | |
| Applicant LUDWIG INSTITUTE FOR CANCER RESEARCH et al. | | |

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 4 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

| | | |
|---|---|---|
| ☒ | Box No. I | Basis of the opinion |
| ☐ | Box No. II | Priority |
| ☐ | Box No. III | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| ☐ | Box No. IV | Lack of unity of invention |
| ☒ | Box No. V | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| ☐ | Box No. VI | Certain documents cited |
| ☐ | Box No. VII | Certain defects in the international application |
| ☒ | Box No. VIII | Certain observations on the international application |

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 11.05.2005 | 23.02.2006 |
| Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465 | Authorized Officer Steffen, P Telephone No. +49 89 2399-7307 |

# INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/003492

## Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

**Description, Pages**

| | |
|---|---|
| 1-31 | as originally filed |

**Claims, Numbers**

| | |
|---|---|
| 1-25 | received on 04.06.2005 with letter of 27.05.2005 |

**Drawings, Sheets**

| | |
|---|---|
| 1/17-17/17 | as originally filed |

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

**Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1,2,4-10,12,14,20-25 |
| | No: | Claims | 3,11,13,15-19 |
| Inventive step (IS) | Yes: | Claims | 1,2,4-10,12,14,20-25 |
| | No: | Claims | 3,11,13,15-19 |
| Industrial applicability (IA) | Yes: | Claims | 1-25 |
| | No: | Claims | |

2. Citations and explanations (Rule 70.7):

**see separate sheet**

**Box No. VIII Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

## Supplemental Box relating to Sequence Listing

**Continuation of Box I, item 2:**

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☒ received by this Authority as an amendment on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

**Re Item V**

**Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

Reference is made to the following documents:

D1: WO 03/000843 A (BRIGHAM & WOMENS HOSPITAL) 3 January 2003 (2003-01-03)

D2: DATABASE EMBL [Online] 27 June 2002 (2002-06-27), STRAUSBERG R.: "Homo sapiens, Similar to protein phosphatase 1, regulatory (inhibitor) subunit 13B, clone IMAGE:4994121, mRNA." XP002307934 retrieved from EBI accession no. EM_PRO:BC032298 Database accession no. BC032298

D3: WO 02/12325 A (LUDWIG INST CANCER RES ; LU XIN (GB)) 14 February 2002 (2002-02-14)

The present application relates to the cloning of a human gene that encodes a protein which inhibits the pro-apoptotic activity of p53 by binding p53 as well as aspects related thereto. The protein is ubiquitinated. A shorter version of this protein, corresponding to the C-terminus of the molecule was described in the prior art. However, although this prior art protein binds to p53, the protein of the present application binds p53 better than the shorter prior art protein, which does bind preferentially to apoptosis inducer Bcl-2. Because of its p53 binding activity the protein of the present application is suitable as modulator of tumour suppression by p53.

D1 discloses the cloning of mouse and human genes encoding a protein being related to p53 binding protein. The protein is involved in dilated cardiomyopathy. With SEQ ID NO: 1, this human protein has 99.5% identity (99.6% ungapped) in 828 aa overlap (1-828:1-827) which corresponds to two amino acid substitutions compared to SEQ ID NO: 1, namely P370S and Δ372L. Vectors, host cells, antibodies and detection methods are also disclosed. Because of these two exchanges, both the genes and proteins are different from the ones claimed in claim 1 and 3, comprising or encoding the sequence of Figure 1a. Moreover, D1 does not discloses antibodies directed to the sequence of amino acids 1-483 in Figure 1a. In conclusion, the amended claims are not affected for novelty by D1.

D2 discloses the cloning of a mRNA as cDNA that is 100% identical to SEQ ID NO: 2. The

RZPD reference in D2 of IRAKp961C1364Q leads to the database entry of RelA-associated inhibitor also called iASPP (that is the above mentioned prior art protein), for which it was shown that it is an inhibitor of p53. The cloning vector in which the gene is inserted is the expression vector pCMV-SPORT6. Also the gene is isolated from tissue type placenta, choriocarcinoma. Therefore and because of the functional implication above an implication in disease and thus usefulness in medicine is self-evident. It is also remarked that ubiquitination is a post-translational modification that is not inscribed in the gene sequence *per se* i.e. the same gene encodes the same protein. Claims 3 and 11 thus lack novelty in view of D2.

D3 discloses a cDNA encoding human apoptosis stimulating protein inhibitor (I-ASP). This sequences (gene and protein) are identical to the sequences of Fig. 2 and there is a complete identity with the 3' end/C-terminus of the sequences presented in Fig. 1 (SEQ ID NO's: 1 and 2). Tests for modulators are described, but not the interaction with Bcl-2 (and I-ASP). Also are antisense molecules for inhibition of gene expression and protein function largely described including medical applications. Since claims 13, 15-19 relate to vectors for the use of inhibiting RNA molecules with antisense molecules, and they are not restricted to the gene part of SEQ ID NO: 2 that is different from the gene of D3, these claims are anticipated by D3. However claim 14 is with its antisense part restricted to the 3' part (N-terminal region) that is not revealed in D3. Therefore this claim is novel over D3.

In summary, claims 3, 11 and 13, 15-19 are anticipated by D1-D3 and thus not novel, contrary to Article 33(2) PCT. At the same time they are not built on inventive step, contrary to Article 33(3) PCT.

Claims 1, 2, 4-10, 12, 14 and 20-25 are novel over D1-D3 because either restricted to polypeptides comprising the sequence of Fig 1a, to antisense nucleic acids restricted to the non-disclosed part of SEQ ID NO: 2, or to non-described interactions (Bcl-2, ubiquitinating enzymes).

From the prior art D1-D3 it was not apparent that a protein with sequence of Figure 1a, that is ubiquitinated existed and that this protein is and effective modulator of p53 activity and binds this latter more efficiently than its truncated version of D3. Moreover was not known or made obvious the fact that the truncated version of the protein of Figure 1a (Figure 2a; D3)

binds to Bcl-2. Therefore the claims for which novelty is recognised are also based on inventive step in accordance with Article 33(3) PCT.

**Re Item VIII**

**Certain observations on the international application**

The claims in general relate to Figures, this is contrary to Rule 6 PCT.

In claim 1 the term "preferentially" is unclear since prone to subjective interpretation (Article 6 PCT).

It is from claims 12 and 13 not clear to what nucleic acid molecule the inhibitory/antisense RNA is directed (e.g. one of the application). In its most largest interpretation a general use of inhibitory/antisense RNA could be encompassed (Article 6 PCT). It appears also that these vectors do not generally show all the characteristics of the claims they are meant to depend on e.g. claims 3 and 11. Thus they do not, despite suggestive wording, depend on these. This is all unclear (Article 6 PCT).

Claim 14 relates to nucleic acids presented in Figure 3. However there are many shown there not all of which are novel sequences. This leads to clarity and possibly novelty problems (Articles 6 and 33(2) PCT).

Claim 16 relates to an undefined "part" of the nucleic acid of the previous claims. This is unclear (Article 6 PCT).

Claims 20, 21, 22 relate to undefined "variants" of p53, Bcl-2 or ubiquitin. This is unclear (Article 6 PCT).

**Claims**

1. An isolated polypeptide wherein said polypeptide comprises the amino acid sequence as shown in Figure 1a, characterised in that said polypeptide has the following characteristics:

   i) a polypeptide which preferentially binds the tumour suppressor polypeptide p53 to inhibit the pro-apoptotic activity of p53 when compared to a polypeptide, or variant thereof, as represented by the amino acid sequence as shown in Figure 2a;

   ii) a polypeptide which includes at least one amino acid residue which residue is ubquitinated.

2. A polypeptide according to Claim 1 wherein said polypeptide consists of the amino acid sequence shown in Figure 1a.

3. A vector comprising a nucleic acid molecule that encodes a polypeptide according to Claim 1 or 2.

4. A method for the production of the polypeptide according to Claim 1 or 2, comprising the steps:

i) providing a cell transformed/transfected with a vector according to Claim 3;

ii) growing said cell in conditions conducive to the manufacture of said polypeptide; and

iii) purifying said polypeptide from said cell, or its growth environment.

5. An antibody, or binding fragment thereof, which binds the polypeptide according to Claim 1 or 2 characterised in that said antibody binds said polypeptide between amino acid residues 1 to 483 of the amino acid sequence shown in Figure 1a.

6. An antibody according to Claim 5 wherein said fragment is a Fab fragment.

7. An antibody fragment according to Claim 6 wherein said antibody is selected from the group consisting of: $F(ab')_2$, Fab, Fv and Fd fragments; and antibodies comprising CDR3 regions.

8. An antibody or binding fragment thereof, according to any of Claims 5-7 wherein said antibody is a humanised.

9. An antibody or binding fragment thereof, according to any of Claims 5-7, wherein said antibody is a chimeric antibody.

10. A polypeptide according to Claim 1 or 2 for use as a pharmaceutical.

11. A vector according to Claim 3 for use as a pharmaceutical.

12 A vector according to Claim 11 wherein said vector encodes an inhibitory RNA molecule.

13. A vector according to Claim 11 wherein said vector encodes an antisense nucleic acid molecule.

14. A vector according to Claim 12 or 13 wherein said antisense molecule or inhibitory RNA molecule is designed with reference to the nucleic acid sequence shown in Figure 3, wherein said antisense or inhibitory RNA molecule is designed to that part of said nucleic acid sequence which encodes amino acid residue 1 to 483 defined as shown in Figure 1a.

15. A vector according to Claim 14 wherein said vector is provided with a transcription cassette comprising a nucleic acid sequence operatively linked to a promoter which promoter transcribes said nucleic acid molecule to produce an antisense nucleic acid molecule, said sequence selected from the group consisting of:

i) a nucleic acid sequence, or part thereof, as represented in Figure 1b;

ii) a nucleic acid sequence which hybridises to the sense sequence presented in Figure 1b and which encodes a polypeptide according to Claim 1 or 2

16. A vector according to Claim 14 wherein said vector is provided with a transcription cassette comprising a nucleic acid molecule, or part thereof, selected from the group consisting of:

i) a nucleic acid molecule represented by the nucleic acid sequence in Figure 1b;

ii) a nucleic acid molecule which hybridises to the sequence in (i) above and which encodes a polypeptide according to Claim 1 or 2; or

iii) a nucleic acid molecule which is degenerate because of the genetic code to the sequences defined in (i) and (ii) above; wherein said cassette is adapted such that both sense and antisense nucleic acid molecules are transcribed from said cassette.

17. A vector according to Claim 16 wherein said cassette is provided with at least two promoters adapted to transcribe both sense and antisense strands of said nucleic acid molecule.

18. A vector according to Claim 17 wherein said cassette comprises a nucleic acid molecule wherein said molecule comprises a first part linked to a second part wherein said first and second parts are complementary over at least part of their sequence and further wherein transcription of said nucleic acid molecule produces an RNA molecule which forms a double stranded region by complementary base pairing of said first and second parts.

19. A vector according to Claim 18 wherein said first and second parts are linked by at least one nucleotide base.

20. A screening method to identify an agent that modulates the interaction of a p53 binding protein with a p53 polypeptide wherein said method comprises the following steps of:

i) forming a preparation comprising a polypeptide according to Claim 1 or 2 and a p53 polypeptide, or sequence variant thereof, and at least on agent to be tested;

i) determining the activity of said agent with respect to the binding of said polypeptide to said p53 polypeptide.

21. A screening method for the identification of an agent which modulates the interaction of a Bcl-2 binding polypeptide with a Bcl-2 polypeptide wherein said method comprises the steps of:

i) forming a preparation comprising a polypeptide as represented by the amino acid sequence shown in Figure 2a and a Bcl-2 polypeptide, or variant thereof, and at least one agent to be tested; and

i) determining the activity of said agent with respect to the binding of said polypeptide to said Bcl-2 polypeptide.

22. A screening method to identify agents which modulate the ubquitination of a polypeptide comprising the steps of:

i) forming a preparation comprising a polypeptide according to Claim 1 or 2, a ubiquitin polypeptide or variant thereof, polypeptide(s) with the specific activity associated with ubiquitin conjugating polypeptides and at least one agent to be tested;

ii) determining the activity of said agent with respect to the conjugation of ubiquitin to said polypeptide.

23. A method according to any of Claims 20-22 wherein said agent is a peptide or polypeptide.

24. A method according to Claim 23 wherein said peptide/polypeptide is an antibody or antibody binding fragment.

25. A method according to any of Claims 20-22 wherein said agent is an aptamer.